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TITLE: TARGETING ENDOTHELIAL METAFLAMMATION TO COUNTERACT DIABESITY CARDIOVASCULAR RISK: CURRENT AND PERSPECTIVE THERAPEUTIC OPTIONS.

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Abstract: The association of obesity and diabetes, termed "diabesity", defines a combination of primarily metabolic disorders with insulin resistance as the underlying common pathophysiology. Cardiovascular disorders associated with diabesity represent the leading cause of morbidity and mortality in the Western world. This makes diabesity, with its rising impacts on both health and economics, one of the most challenging biomedical and social threats of present century. The emerging comprehension of the genes whose alteration confers inter-individual differences on risk factors for diabetes or obesity, together with the potential role of genetically determined variants on mechanisms controlling responsiveness, effectiveness and safety of anti-diabetic therapy underlines the need of additional knowledge on molecular mechanisms involved in the pathophysiology of diabesity. Endothelial cell dysfunction, resulting from the unbalanced production of endothelial-derived vascular mediators, is known to be present at the earliest stages of insulin resistance and obesity, and may precede the clinical diagnosis of diabetes by several years. Once considered as a mere consequence of metabolic abnormalities, it is now clear that endothelial dysfunctional activity may play a pivotal role in the progression of diabesity. In the vicious circle where vascular defects and metabolic disturbances worsen and reinforce each other, a low-grade, chronic, and 'cold' inflammation (metaflammation) has been suggested to serve as the pathophysiological link that binds endothelial and metabolic dysfunctions. In this paradigm, it is important to consider how traditional antidiabetic treatments (specifically addressing metabolic dysregulation) may directly impact on inflammatory processes or cardiovascular function. Indeed, not all drugs currently available to treat diabetes possess the same anti-inflammatory potential, or target endothelial cell function equally. Perspective strategies pointing at reducing metaflammation or directly addressing endothelial dysfunction may disclose beneficial consequences on metabolic regulation. This review focuses on existing and potential new approaches ameliorating endothelial dysfunction and vascular inflammation in the context of diabesity.

Keywords: endothelial dysfunction; diabesity; metaflammation; anti-diabetic drugs

Conflict of interest The authors declare no conflict of interest.

SUMMARY

DIABESITY

ENDOTHELIAL DYSFUNCTION AND METAFLAMMATION

- *Metabolic derangement triggers endothelial dysfunction*
- *Endothelial dysfunction contributes to metabolic abnormalities*
- *Inflammatory signaling links endothelial to metabolic impairments*

HOW CONVENTIONAL DIABETIC TREATMENTS MAY AMELIORATE ENDOTHELIAL DYSFUNCTION

- *Metformin*
- *Thiazolidine-2-4-diones (TZDs)*
- *Glucagon-like peptide-1 receptor (GLP-1R) agonists*
- *Dipeptidyl peptidase 4 (DPP4) inhibitors*

NEW STRATEGIES FOR TREATMENT OF DIABETES AND THEIR IMPACT ON ENDOTHELIAL DYSFUNCTION

- *Anti-inflammatory drugs*
- *NLRP3 inflammasome inhibitors*
- *AGE inhibitors*
- *PKC inhibitors*
- *VEGF inhibitors*
- *PARP inhibitors*
- *ROCK (Rho-associated kinase) inhibitors*
- *AMPK activators*
- *Anti-oxidants*
    - *Vitamin C and Vitamin E*
    - *Polyphenols*

CONCLUSIONS

DIABESITY

Diabetes occurring in the context of obesity has been defined "diabesity" [1] and represents a worldwide growing phenomenon affecting both developed and developing countries (https://ec.europa.eu/research/health/pdf/diabesity-conference-report-022012_en.pdf). The social and economic burden of diabesity includes consequences in terms of productivity and life expectancy, costs related to health care once the disease has been diagnosed, and costs connected with long-term complications such as blindness, limb amputation, or kidney and heart diseases.

The association between diabesity and cardiovascular risk is well recognized, and increasing attention has been devoted to the long-term effects of drug treatments on progression and severity of cardiovascular morbidity and mortality. Current therapeutic options involve agents that target elevated blood sugar, impaired insulin resistance, increased blood pressure, and high cholesterol levels. Nevertheless, these abnormalities represent downstream symptoms more than causal agents of diabesity, with the consequence that a significant number of monotherapy treatments lacks efficacy overtime and/or does not adequately control cardiovascular complications [2]. In addition, inappropriate therapies can increase the risk of hypoglycemic episodes, which in turn may trigger cardiovascular acute events.

In the clinical practice, therapeutic recommendations to treat metabolic and cardiovascular disturbances are largely based on standard protocols that address typical dysfunctions in average diabetic and obese patients. Unfortunately, this approach may be unsuccessful or inappropriate in subjects that, for a variety of reasons, are unable to reach the therapeutic goals. One of these reasons is based on the recognition that both obesity and diabetes are multifactorial diseases, resulting from the complex interplay between environmental factors and genetic inheritance. Therefore, diabetic and obese patients may differ in their individual susceptibility to the disease or their response to a specific treatment.

Significant progresses have been made in understanding the variant genes predisposing to these diseases [3]. At present, the high susceptibility makes the predictive value of the gene variants very limited. Nevertheless, novel scientific discoveries at the genomic level are expected to shed light on risk factors for diabetes or obesity, and help to identify subpopulations of patients with specific characteristics.

On the same line, the increased appreciation of the inter-individual differences in response to drugs have highlighted the potential role of genetically determined variants on mechanisms controlling the absorption, bioavailability, tissue responsiveness, effectiveness and safety of current anti-diabetic therapy [4,5]. Although the clinical applicability of these data requires further efforts, the evolving area of pharmacogenomics and pharmacogenetics opens the road to the possibility of a more individual-tailored, personalized medicine [6,7].

Considering the economic impact of diabesity, it is of foremost importance to plan strategies and approaches that may enhance prediction on the onset and course of the disease, and at the same time identify individuals who are most likely to benefit from a specific management strategy. In this complex scenario, the rising number of options to treat diabesity significantly broadens the range of therapeutic opportunities, but concomitantly underlines the need of additional knowledge on molecular mechanisms involved in the pathophysiology of diabesity. A state of chronic, low-grade inflammation in which inflammatory molecules produced by infiltrating macrophages exert pathological changes in all insulin-sensitive tissues has been proposed to bridge the gap between epidemiology and pathobiochemistry of diabesity.

The role played by the endothelium in triggering and/or enhancing metaflammation is particularly intriguing, and sets a new challenge on the development of novel therapies to treat diabesity. In addition, endothelial dysfunction is - to a certain extent- a reversible process, and ameliorated endothelial function is indicative of improved cardiovascular protection. Along with the implications related to its potential role on cardiovascular risk, evaluation of endothelial function might represent a useful biomarker to assess the effectiveness of therapy. Current techniques to measure endothelial function do not fulfill all the essential criteria required for a clinical surrogate

end-point and, at present, brachial artery flood mediated dilation (FMD) remains the most widely applied non-invasive method. Nevertheless, the importance of endothelium in participating and predicting cardiovascular risk factors encourages further efforts in this direction. In the next paragraphs the potential contribution of endothelial dysfunction to metaflammation and metabolic disturbances of diabesity is briefly discussed.

ENDOTHELIAL DYSFUNCTION and METAFLAMMATION

With the growing understanding of the functional role played by the endothelium, and the subsequent discovery of several endothelial mediators and their respective mechanism of action, it has become increasingly clear that endothelial abnormalities represent an early sign of both hemodynamic and metabolic disturbances [8]. Indeed, endothelial dysfunction - a condition in which the endothelium loses its physiological properties and shifts toward a vasoconstrictor, pro-thrombotic and pro-inflammatory state - is considered a major contributing factor in the etiology of diabetes-related microvascular diseases such as retinopathy, nephropathy, neuropathy, and impaired wound healing [9,10]. Moreover, endothelial dysfunction precedes the onset of macrovascular complications, mainly represented by atherosclerosis, which in diabetic patients is more rapid and more severe than in control population [10,11].

Typically, endothelial dysfunction is defined by a reduced availability of nitric oxide (NO), a gaseous mediator with vasodilatant, anti-thrombotic and anti-inflammatory properties [12,13]. NO bioavailability depends on several multiple factors, ranging from the efficiency of the producing enzyme endothelial NO synthase (eNOS) to the speed of conversion of NO itself to more stable nitrate/nitrite derivatives. The dynamic, highly regulated physiological production of NO in the endothelium may be disrupted when eNOS protein expression is decreased, when eNOS substrates and/or co-factors are insufficient, when enzymatic activity of eNOS is impaired or uncoupled, or when the production of endothelial mediators with opposing vascular effects is relatively increased [9,14]. The deficiency of NO bioavailability and the increased reactive oxygen species (ROS) and proinflammatory factors are requisite hallmarks for endothelial dysfunction [15].

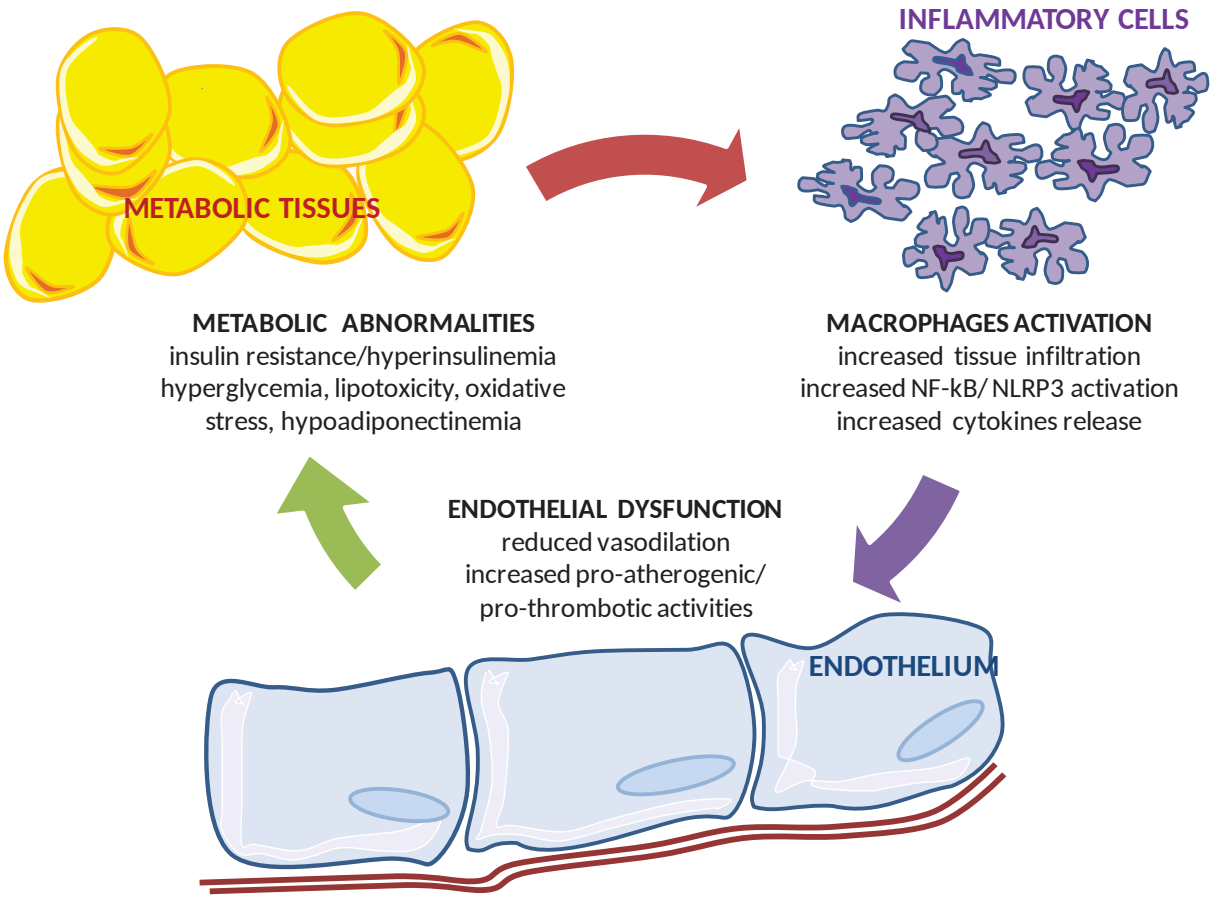

Figure 1. The vicious circle linking metabolic abnormalities and inflammatory signaling to endothelial dysfunction in diabetes.

In the reciprocal relationship between metabolic abnormalities and vascular dysfunction, a key role has been attributed to inflammation: when endothelial cells undergo inflammatory activation by cytokines such as IL-1β and TNF-α, or by ox-LDL uptake via ox-LDL receptor-1 (LOX-1) this results in the increased expression of selectins and adhesion molecules that promote the adherence of monocytes. In turn, continued release of cytokines, such as MCP-1, by activated endothelial cells not only perpetuate inflammation but also contribute to lipid accumulation within the atheroma and dysregulated activity of underlying vascular smooth muscle cells (see [16] for review). The most significant mechanisms linking metabolic abnormalities, inflammatory signaling and endothelial dysfunction in diabetes are summarized below and schematically depicted in Figure 1.

Metabolic derangement triggers endothelial dysfunction - Metabolic derangements known to occur in diabetes include, among others, hyperglycemia, oxidative stress, excess free fatty acid release, and lipotoxicity, with insulin resistance and compensatory hyperinsulinemia underlying and driving all others. Each of these abnormalities results from complex rearrangement of physiological homeostasis and may impact on endothelial function individually [17,18], and/or participating in a vicious cycle, where every change worsen and reinforce all others [19]. The specific molecular mechanisms by which these disturbances disrupt NO synthesis or degradation, and their overall impact on endothelial function has been deeply investigated over time and extensively debated elsewhere [9,20].

One fundamental notion that helps to explain the tight link between diabetic metabolic abnormalities and vascular impairment is the well-recognized effect of several metabolic mediators

on hemodynamic homeostasis. Direct vascular effects have been documented, in addition to insulin [21], for gonadal steroids [22], for hormones like leptin [23] and ghrelin [24], and for a number of bioactive proteins secreted by adipose tissue and skeletal muscles termed "adipokines" and "myokines", respectively [25]. In the context of diabetic endothelial pathophysiology, the cardiovascular properties of insulin and adiponectin (Ad) are perhaps the most relevant in terms of current and perspective therapeutic approaches [26].

Insulin - Biological vascular actions of insulin have been clearly identified and progressively described among the last three decades (comprehensively described in [21]). In addition to systemic hemodynamic effects, related to renal reabsorption of sodium, or stimulation of sympathetic activity, insulin is capable to directly modulate endothelial release of both vasoconstrictor factors such as endothelin-1 (ET-1) and vasodilating mediators including NO. All these activities physiologically contribute to overall metabolic homeostasis [27], since insulin-mediated activation of eNOS and subsequent production of NO enhance local vasodilation, blood flow and nutrient delivery, thereby participating to efficient insulin-mediated glucose uptake on target tissues [28]. Production of NO in response to insulin depends on activation of insulin receptor tyrosine kinase (IR), and involves a signaling cascade leading to phosphorylation of eNOS on Ser^{1177} via a signaling pathway recruiting insulin receptor substrate-1 (IRS-1), phosphatidylinositol (PI) 3-kinase and Akt [29-33]. Concomitantly, the insulin-dependent activation of the Ras/MAP kinase signaling stimulates secretion of ET-1 from endothelial cells [34-36], and it is involved in insulin-stimulated expression of adhesion molecules including VCAM-1 and E-selectin [37]. Thus, by activating distinct intracellular signaling pathways, insulin modulates the endothelial production of mediators with opposing vascular effects. It is of particular interest that endothelial signaling pathways related to production of NO and metabolic signaling pathways regulating translocation of the glucose carrier GLUT4 are almost completely overlapping. Common insulin signaling in distinct tissues with metabolic or vascular functions helps to understand why a selective impaired sensitivity in the PI-3K/Akt signaling branch may greatly contribute to the pathophysiology of diabetic vascular complications [19]. On this respect, the emerging comprehension of the genes whose alteration confers an elevated risk to develop diabetes and insulin resistance might also help to predict the onset of insulin-dependent endothelial dysfunction: for example, polymorphisms in the IRS-1 gene (associated to impaired PI 3K binding and insulin secretion in the beta-cells) [38] might also predispose to defects in insulin-mediated NO production in endothelium. Similarly, genetic defects in the IR gene or in genes encoding downstream signaling proteins may affect vascular actions of insulin as well as metabolic activity in target tissues (extensively reviewed in [3]). In the next years, further analysis of results from the genome-wide association studies (GWAS) will be important to uncover additional gene variants and, hopefully, to turn these information into the identification of prognostic and predictive biomarkers of insulin resistance. In the context of diabesity, this would be of utmost importance since insulin-dependent endothelial dysfunction is known to precede and predict the onset of metabolic abnormalities.

Adiponectin - Adiponectin (Ad) is a protein hormone produced exclusively by adipose tissues with identified beneficial effects on insulin sensitivity and lipid metabolism [39]. In addition, Ad exerts multiple vasoprotective effects via its anti-inflammatory, antioxidant, antiapoptotic, antiatherogenic, vasodilatory, and antithrombotic properties on endothelial cells, monocytes, macrophages, leukocytes, platelets, and vascular smooth muscle cells [40]. The gene encoding Ad is located in the chromosome region 3q27, a locus mapped for susceptibility to both diabetes and cardiovascular risk factors. Among several single nucleotide polymorphisms (SNPs) examined in the Ad gene, SNPs at positions 45 [41,42] and 276 [43] have been linked to increased risk of cardiovascular events in diabetic individuals. These findings support the influence of Ad genetic variability on cardiovascular protection, especially in patients with diabetes [44]. Circulating Ad may exist as monomers, or form oligomers and multimers. The exact role for each of these multiple Ad conformations is not completely defined, but the high-molecular-weight (HMW) Ad is particularly active and equally able to bind both the AdipoR1 and the AdipoR2 to exert metabolic and vasoprotective effects in target

tissues [45]. On endothelial cells, Ad binding to AdipoR1/2 receptors activates AMP kinase (AMPK), that leads to an increase in eNOS activity and NO production via phosphorylation of eNOS at Ser^{1177} and Ser^{633} [46]. Other beneficial endothelial effects of Ad include its ability to counteract the expression of adhesion molecules and to suppress the TNF-α-mediated monocyte adhesion [47]. The signaling events linking AdipoR1/2 receptors to activation of AMP kinase/eNOS require, among others, the adaptor protein APPL1 [48], but the exact sequence of receptor downstream signaling cascade has not been completely elucidated. Nevertheless, APPL1 isoform is involved in cellular mechanisms resulting in Ad-suppressed foam cell formation [49], suggesting that the Ad-AdipoR1/2-APPL1 axis may be a potential therapeutic target for preventing endothelial activation and atherosclerotic processes. Interestingly, while production and release of proinflammatory cytokines increases proportionally to adipose mass under obesity, Ad levels are inversely reduced, and hypoadiponectinemia has been is considered a significant predictor of endothelial dysfunction in both the peripheral and coronary arteries besides other markers of the metabolic syndrome [50]. These clinical observations are supported by experimental findings showing that Ad-deficient animals are more prone to develop neointimal hyperplasia [51], impaired endothelium-dependent vasodilation [52], and high blood pressure [53]. Accordingly, exogenous administration of Ad has been effective to improve cardiovascular disease in animal models. At present, however, a number of concerns limit the possibility to extend this approach to humans: these include the high circulating levels needed, the unclear role of monomers, oligomers and multimers of Ad, and its moderately short half-life, together with the need to better characterize the extensive posttranslational modifications that might affect its potential beneficial activity in patients. In the meantime, interventions aiming at increasing the endogenous Ad levels or restoring tissue sensitivity to Ad may represent potential alternatives to prevent diabetes-related vascular disorders. On this respect, several nutraceutical compounds have been shown to enhance plasma Ad levels, including green tea extract [54] and resveratrol [55]. Similarly, a number of currently used pharmacological agents such as renin-angiotensin system blockers, bezafibrate and fenofibrate, thiazolidinediones, statins, and nebivolol are able to increase plasma Ad levels [40,56]. Although the contribution of Ad to overall effects of these drugs is not always straightforward, these findings altogether support the possibility to consider circulating Ad levels not only a biomarker of vascular disturbances under diabesity, but also an indicator of the effectiveness for specific treatments. Currently, total serum and urinary Ad levels may be measured by commercially available radioimmunoassay kit or enzyme-linked immunoadsorbent assays. However, laboratory methods still lack appropriate standardization, and the determination of ideally therapeutic Ad levels for each clinical setting requires further efforts [57].

Endothelial dysfunction contributes to metabolic abnormalities - The recognition that metabolic and endothelial physiology are fully integrated and reciprocally controlled suggests that, beside representing the immediate target of metabolic abnormalities, unbalanced endothelial function may in turn actively contribute to disrupted metabolic homeostasis [58]. This has long been hypothesized for angiotensin II [59], and more recently demonstrated for prostanoids [60] and NO itself [61]. The interference of ET-1 on metabolic function has been deeply investigated: ET-1 infusion results in hyperinsulinemia and insulin resistance in vivo [62], and chronically elevated ET-1 levels may contribute to desensitization of metabolic signaling pathways on adipocytes [63]. In addition, ET-1 inhibits adipocyte differentiation, reduces lipoprotein lipase activity, inhibits insulin-stimulated glucose uptake [64], and stimulates lipolysis [65]. ET-1 seems involved in production and secretion of Ad [66]. On this respect, we have recently provided evidence that increased circulating levels of ET-1 in obese children directly contribute to reduced levels of Ad via ET receptor-mediated activation of p42/44 MAPK signaling pathways [67].

The relationship between endothelial dysfunction and metabolic derangement is further complicated by the limited ability of endothelium to regenerate overtime. Physiologically, endothelial cell injury is at least partially mitigated by endogenous reparative processes mediated by bone marrow-derived endothelial progenitor cells (EPC) [68]. Under chronic exposure to damaging

events, however, EPC availability and/or mobilization tends to progressively decrease, and this may accelerate the onset of endothelial dysfunction [69]. Insulin resistance reduces EPCs survival and diminish their capacity for adhesion, endothelial integration, proliferation, and differentiation by multiple mechanisms [70] (see [71,72] for review). These involve, among others, a reduced NO bioavailability associated to increased oxidative stress and impaired PI 3K/Akt signaling, which are found to alter the cytoskeletal structure and decrease mobilization of EPCs in diabetic and obese patients [73-75]. Based on these data, EPCs have been proposed as cellular biomarkers of disease and predictors of cardiovascular outcomes. An updated definition of their role and their biological properties in health and disease has been recently published [76].

Inflammatory signaling links endothelial to metabolic impairments – In diabetes, glucotoxicity and lipotoxicity induce a proinflammatory trait in macrophages residing or invading the adipose tissue and the vasculature [77,78], and are responsible for oxidative and endoplasmic reticulum stress. This in turn elicits the activation of thioredoxin-interacting protein (TXNIP) and the NLR family, pyrin domain containing 3 (NLRP3) inflammasome, which increase the release of active interleukin (IL)-1β [79,80]. IL-1β-amplified inflammation increases the expression of various cytokines and chemokines, and favors the recruitment of macrophages in diabetic β-cells, adipose tissue, and blood vessels [19,78]. In turn, the unbalanced activity in endothelium may further enhance leukocytes adhesion, and increased release of inflammatory cells, thereby promoting lipids deposition and facilitating the atherosclerotic plaques formation. In addition, alterations in the gut microbiome along with increased gut leakiness of bacterial wall lipopolysaccharides (endotoxins) may further promote tissue inflammation [81]. Endotoxins, free fatty acids, and cholesterol induce inflammation by activating Toll-like receptor (TLR) pathways and, subsequently, nuclear factor-κB (NF-κB)-mediated release of a broad range of cytokines and chemokines including tumor necrosis factor (TNF), IL-1β, IL-8, and MCP-1 that promote the accumulation of various immune cells in different tissues [77,78].

The likelihood that blockade of vascular inflammation and oxidative stress may be effective to prevent metabolic disorders is supported by the finding that suppression of inflammatory processes in the vasculature prevents the onset of insulin resistance in metabolic target tissues and prolongs lifespan [82]. In a transgenic animal model, overexpression of the inhibitory NF-kB subunit IkBα in endothelium correlates with reduced macrophage infiltration in adipose tissue and decreased circulating markers of oxidative stress, concomitantly increasing blood flow, muscle mitochondrial content, and locomotor activity [82]. These findings confirm the pivotal role of the transcription factor NFkB in oxidative stress, vascular dysfunction, and inflammation, and further support the central role of endothelium in obesity-induced insulin resistance.

On the other hand, proinflammatory cytokines may play important roles in the pathogenesis of both endothelial dysfunction and insulin resistance [83]. Development of insulin resistance has classically been attributed to adipocyte-derived inflammation, resulting in macrophage infiltration and altered secretory profile of adipose tissue depots with increased levels of pro-inflammatory cytokines and decreased Ad synthesis and release [84-86]. The differential role of regional fat depots in synthesis and release of specific mediators and vasoactive substances may help to reconcile the generally accepted 'outside-in' theory of vascular inflammation, which postulates that inflammation begins in adipose tissue and then spreads inward to the vasculature [87], with the 'inside-out' process of vascular inflammation, proposing that the first step is intimal injury, that then extends to the media and adventitia [88,89]. For example, it is plausible that intimate connections between perivascular adipose tissue (PVAT) and other components of the vessel wall result in PVAT being the first adipose depot to sense and respond to signals from circulating bioactive factors that influence activity of endothelial and vascular smooth muscle cells [90-92].

On this regard, another important inflammatory pathway, the NLRP3 inflammasome, has been proposed to be involved in the vasculoprotective effects exerted by Ad [93]. The NLRP3 inflammasome is a large multimeric protein complex mediating the cleavage of inactive

prointerleukin- (IL-) 1β and IL-18 into their active form [94]. We have recently provided evidence that activation of NLRP3 inflammasome contributes to the development of insulin resistance and diet-induced renal and myocardial dysfunctions, mainly by inducing IL-1β and IL-18 overproduction [95-97]. Activation of the endothelial NLPR3 inflammasome by injurious adipokines such as visfatin may disrupt inter-endothelial junctions and increase paracellular permeability of the endothelium contributing to the early onset of endothelial injury during metabolic disorders [98]. The role of NLRP3 inflammasome in evoking tight junction disruption induced by high glucose has been demonstrated both in vivo and in vitro, resulting in increased release of the high mobility group box protein-1 (HMGB1), which enhances permeability of endothelial monolayers possibly via its autocrine or paracrine action on receptor for advanced glycation end products (RAGE)-mediated pathway [99]. Using established mouse models of coronary arteritis, recent studies have implicated NLRP3 inflammasome as a major intracellular molecular machinery able to switch on the inflammatory responses that contribute to the development of endothelial dysfunctions leading to atherosclerotic acceleration [100, Tomita, 1993 #125,101,102]. Such activation of the endothelial NLPR inflammasome is due to lysosome membrane permeabilization and cathepsin B release and can be suppressed by lysosome stabilization agents [103]. Thus, the Ad-induced inhibition of NLRP3 inflammasome activation, as recently documented [104] may represent an original and pivotal cross-talk mechanism to mitigate endothelial dysfunctions due to metabolic derangements.

HOW CONVENTIONAL DIABETIC TREATMENTS MAY AMELIORATE ENDOTHELIAL DYSFUNCTION

Current therapeutic options to treat type 2 diabetes aim at reducing plasma glycemic levels by increasing insulin pancreatic secretion and/or ameliorating insulin sensitivity in peripheral tissues. Although blood glucose normalization and increased insulin sensitivity may *per se* prevent endothelial dysfunction and reduce low-grade inflammation via indirect interrelated mechanisms, a growing number of clinical studies have been conducted to ascertain the specific effects on endothelial function and inflammatory signaling pathways for most of the drugs used (see [105,106] for review). Beside insulin and its analogues (reviewed in [107]), conventional anti-diabetic drugs include biguanides compound metformin, insulin secretagogues, insulin sensitizers, and inhibitors of the alpha-glycosidase. Incretin analogs such as glucagon-like peptide 1 (GLP-1) agonists and dipeptidyl peptidase-4 enzyme (DPP4) inhibitors represent novel classes of anti-diabetics [108,109]. Finally, sodium-glucose co-trasporter-2 (SGLT-2) inhibitors have been more recently introduced on the market and successfully included among therapeutic options to treat diabetes [110,111].

Although extensive revision of vascular effects for all conventional anti-diabetic drugs is beyond the scope of this review, metformin – despite being the oldest drug used - deserves some attention for its multiple effects on endothelial and vascular cells, and for its potential "anti-inflammatory" activities. In addition, we briefly describe thiazolidinediones, whose specific effects on endothelial function have prompted the current recommendation to explicitly evaluate cardiovascular effects for all compounds investigated for diabetes. The vascular profile of incretins and DPP4 inhibitors, and the profile of SGLT-2 inhibitors in terms of endothelial and vascular function is also introduced (Figure 2).

Metformin - This synthetic dimethyl biguanide has been in clinical use for over 55 years. Despite almost 6 decades of research, the cellular mechanisms that underlie the cardioprotective effects of metformin are not completely understood, but a number of clinical studies report an improved endothelial function associated to increased flow-mediated vasodilatation in patients treated with metformin (reviewed in [112]). On one side, the vascular protective actions of metformin are thought to be secondary to the antihyperglycemic effects of this drug, mediated via activation of adenosine 5′-monophosphate–activated protein kinase (AMPK) and subsequent inhibition of hepatic gluconeogenesis, fatty acid oxidation and insulin sensitizing action in striated muscle and adipose tissue [113]. On the other hand, data from both clinical and bench studies

indicate that metformin has a direct action on the endothelium that seem to involve a reduction in oxidative stress secondary to modulation of mitochondrial complex 1, and activation of signaling pathways controlled by the deacetylase Sirtuin 1 (SIRT-1) [114]. In addition, metformin possesses anti-inflammatory effects secondary to inhibition of cytokine-induced activation of NF-κB-mediated pathways in endothelial cells [115], and it has been shown to enhance eNOS activation via an AMPK-dependent signaling [116,117]. Activation of AMPK/eNOS signaling by metformin seems also responsible for the ameliorated angiogenic function of bone marrow-derived EPC [118,119]. Pharmacogenetic studies have suggested that therapeutic responses to metformin may differ inter-individually due to some polymorphisms in the genes encoding for organic cation transporters (OCTs), responsible for metformin active transport across membranes into the intestinal epithelial cells, hepatocytes and renal tubular cells. This may result in metformin intolerance in some subjects, while in others might explain the reduced efficacy of this drug in controlling both metabolic and vascular disturbances [120].

Thiazolidine-2-4-diones (TZDs) – These insulin sensitizer agents are exogenous activators of the peroxisome proliferator-activated receptor-γ (PPAR-γ) [121]. TZDs may improve endothelial function, increase forearm blood flow and reduce blood pressure in humans by both direct and indirect mechanisms (reviewed in [122]). In endothelial cells, TZDs-mediated improvement of PI 3-K signaling pathways results in increased protein expression of eNOS [123] and subsequent enhanced production of NO in response to insulin and other mediators acting via PI 3-kinase-dependent pathways [124]. TZDs may also improve eNOS activity and NO bioavailability by decreasing eNOS uncoupling, reducing generation of superoxide anion [125] and diminishing ADMA levels in vessels from diabetic patients [126]. Among TZDs, rosiglitazone has been shown to counteract hyperglycemia-mediated oxidative stress not by a PPARγ activation mechanism, but rather as a consequence of AMPK-dependent signaling pathways in endothelium [127]. Activation of AMPK in response to rosiglitazone correlates with inhibition of the DAG/PKC pathway, subsequent reduction of NADPH oxidase activity, and amelioration of oxidative balance [127].

Anti-inflammatory effects of TZDs on NF-kB/STAT/AP-1 signaling pathways correlate with a marked decrease in the inducible NOS isoform (iNOS) expression, which in turn decreases the amount of reactive oxygen species produced in monocyte/macrophages and in target tissues of metabolic derangements [128-131]. Moreover, both pioglitazone and rosiglitazone reduce plasma levels of TNF-α, leptin, PAI-1 and C-reactive protein, decrease vascular expression of adhesion molecules, and significantly improve circulating levels of Ad [132]. These activities, associated to the reduced expression of matrix metalloproteases-9 (MMP-9) [133], decreased levels of both FFA and atherogenic LDL cholesterol particles [134], and reduced PAI-1 plasma levels, may contribute to maintain plaque stability, prevent platelet aggregation and inhibit thrombus formation. The significant decrease in systolic blood pressure (SBP) observed in diabetic patients treated with TZDs further supports their ability to improve endothelial function [135].

Thus, when the first meta-analysis reported a significant increase in myocardial infarction (MI) and in cardiovascular-related risk death (CVD) in patients assuming rosiglitazone [136], the long list of apparently beneficial effects of TZDs on endothelial cells and vascular function generated a rather confusing reaction [122]. Whether the puzzling results were related to the single drug rosiglitazone or to the whole class of TZD is still an incompletely resolved question. However, single nucleotide variations found in PPAR-γ gene might, at least in part, help to explain the different therapeutic outcome in patients treated with TZDs [137]. Analogously, variants of the gene encoding for CYP2C8 hepatic enzyme may impair rosiglitazone clearance and hence contribute to the degree of therapeutic/harmful effects of TZDs in particular subjects [138]. While the European Medicine Agency (EMA) recommended withdraw of all licensed rosiglitazone-containing drugs in 2010, the FDA opted for rosiglitazone use restriction in the US [139]. However, following the rosiglitazone lesson, from 2008 all drugs investigated for diabetes must also undergo specific clinical trials evaluating their cardiovascular safety profile.

Glucagon-like peptide-1 receptor (GLP-1R) agonists - Incretins are peptides produced by the gastrointestinal system able to enhance insulin secretion in a glucose-dependent manner [140]. The two main human incretins are glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) whose secretions are impaired in diabetes. GLP-1-mediated effects may be sustained and improved with two strategies currently available: longer-acting GLP-1 receptor agonists resistant to degradation of the dipeptidyl peptidase 4 (DPP4) enzyme, that can provide supraphysiological stimulation of GLP-1 receptor [141]; and inhibitors of DPP4 enzyme, that extends the half-life of endogenous GLP-1 [142].

The receptor for GLP-1 (GLP-1R), originally identified in pancreatic cells, is also expressed on endothelial cells, cardiomyocytes and coronary smooth muscle cells [143]. Activation of GLP-1R improves myocardial function, improves endothelial function in high risk cardiac patients, and enhances natriuresis, with potential positive implications for systolic blood pressure control [144]. Beneficial effects of GLP-1 on endothelial cells are mediated by an increased NO production, and in endothelial cells from human coronary arteries GLP-1R agonists stimulate proliferation via eNOS-, PKA-, and PI3K/Akt-dependent pathways [145]. It has also been shown that GLP-1 exerts a protective effect on atherosclerosis by reducing neointimal formation [146], foam cell formation, and atherosclerotic lesion size [147].

The GLP-1 receptor agonists (GLP-1Ra) are peptides and, such as insulin, require subcutaneous injection to avoid degradation by gastrointestinal enzymes. Most of the clinical trials, in line with animal studies, have reported a reduction of blood pressure after chronic treatment of GLP-1R agonists [148-151]. This may be explained, at least in part, by the ability of GLP-1 analogs to upregulate anti-oxidative enzymes and inhibit NFkB-mediated inflammatory signaling on endothelial cells [152,153], and to reduce oxidative stress by suppressing MAPK signaling pathways in peripheral lymphocytes of type 2 diabetic patients [154]. GLP-1-associated improvement of several cardiovascular markers suggests that therapies with GLP-1R agonists may have a positive effect on cardiovascular risk factors in patients with diabetes. Nevertheless, their long-term safety profile and the direct clinical benefit to cardiovascular outcomes remain to be determined [155]. Pharmacogenetic studies on this class of drugs are still limited.

Dipeptidyl peptidase 4 (DPP4) inhibitors - DPP4 is a widely expressed membrane serine exopeptidase involved in degradation of various oligopeptides, including GLP-1. DPP4 is anchored to the cell membrane but, under certain circumstances, may be released in soluble form. Endothelial cells are the main source of soluble DPP4 form, whose expression and enzymatic activity may increase after chronic exposure to high glucose concentrations [156].

Currently approved DPP4 inhibitors include sitagliptin, saxagliptin, linagliptin and vildagliptin [157]. These small molecular-weight substances inhibit more than 90% of DPP4 activity and can be orally administered. Several studies in animal models support the evidence of DPP4 inhibition in improving endothelial function and blood pressure [158,159]. In isolated aorta rings incubated with DPP4-inhibitor, the relaxant effect of DPP4-inhibitor is GLP-1 independent and results from Akt posphorylation and eNOS activation with a rapid increase in NO levels [160]. Saxagliptin treatment has been shown to reduce blood pressure levels in the spontaneously hypertensive rats with a concomitant increase of aortic and glomerular NO release and comparable reductions in peroxynitrite levels [161]. There is good evidence that DPP4 inhibition mediates protective effect on myocardial infarction, hypertension and atherosclerosis. Pharmacological treatment with sitagliptin has been able to enhance the expression of cardioprotective proteins and improve heart functional recovery after I/R injury [162]. It is not clear how these potential benefits may be mediated, but one possibility involves the DPP4 inhibitors ability to modulate innate and adaptative immunity by suppressing the NF-kB signaling downstream TNF and IL-6 [143,159], and by inhibiting the NLRP3 inflammasome, a multiprotein complex involved in caspase-1 activation and downstream maturation of pro-inflammatory cytokines, TLR4 and IL1β in human macrophages [163].

In contrast to the positive effects in animal experiments, the consequences of treatment with DPP4 inhibitors on endothelial functions in humans have not always been consistent. In a double-blind study on patients with diabetes, treatment with vidagliptin for 4 weeks improved forearm blood flow in response to intra-arterially delivered acetylcholine [164]. On the contrary, other studies measuring FMD of the brachial artery have shown diametrically opposite results, suggesting that sitagliptin and alogliptin actually seem to worsen flow-mediated dilation (FMD) when used to treat diabetic patients [165]. At present, clinical evidence supporting the vascular protective effects of gliptins is uncertain, given the relatively short follow-up [166-168]. Future and ongoing studies (CAROLINA, http://clinicaltrials.gov/show/NCT01243424; TECOS, https://clinicaltrials.gov/show/NCT00790205) should help determine whether DPP4-inhibitors may contribute to improve cardiovascular outcomes in patients with T2DM.

Sodium glucose co-transporter 2 (SGLT-2) inhibitors. The isoform 2 of the sodium glucose transporter (SGLT-2) is located in the proximal convoluted tubule of the kidney and it reabsorbs approximately the 90% of filtered glucose. Inhibition of SGLT-2 represents a novel strategy for achieving glucose control in diabetic patients. It is based on a mechanism of action that targets the kidney to promote urinary glucose excretion and reduce hyperglycemia, and is therefore independent of pancreatic β-cell function or the degree of insulin resistance [169,170].

Among the SGLT-2 inhibitors, canagliflozin, dapagliflozin, and empagliflozin are currently used. Other gliflozins include ertugliflozin and sotagliflozin and, in Japan, ipragliflozin, tofogliflozin and luseogliflozin. Most of clinical trials of SGLT-2 inhibitors show that patients receiving dapagliflozin, canagliflozin, or empagliflozin as add-on therapy exhibit reductions in systolic blood pressure of approximately 3-5 mmHg versus placebo [171,172]. One possible explanation is that urinary glucose excretion stimulated by inhibition of SGLT-2 causes a diuretic effect responsible for lowering blood pressure levels. However, a recent study on dapagliflozin proposed that SGLT-2 inhibitors may possess an additional diuretic-like capacity to lower blood pressure [173]. The improvements in blood pressure, associated to a moderate decrease in body weight [174] induced by treatment with SGLT-2 inhibitors suggest the potential for reducing the risk of cardiovascular events [175,176]. To date, SGLT-2 inhibitors are generally well tolerated, with a favorable safety profile similar to that of placebo. As easily expected, common adverse effects of SGLT-2 inhibitors include genital tract infections and osmotic diuresis. Results from large cardiovascular trials underway for dapagliflozin, canagliflozin and empagliflozin will likely provide greater insights into the effects of SGLT-2 inhibition on cardiovascular outcomes [177].

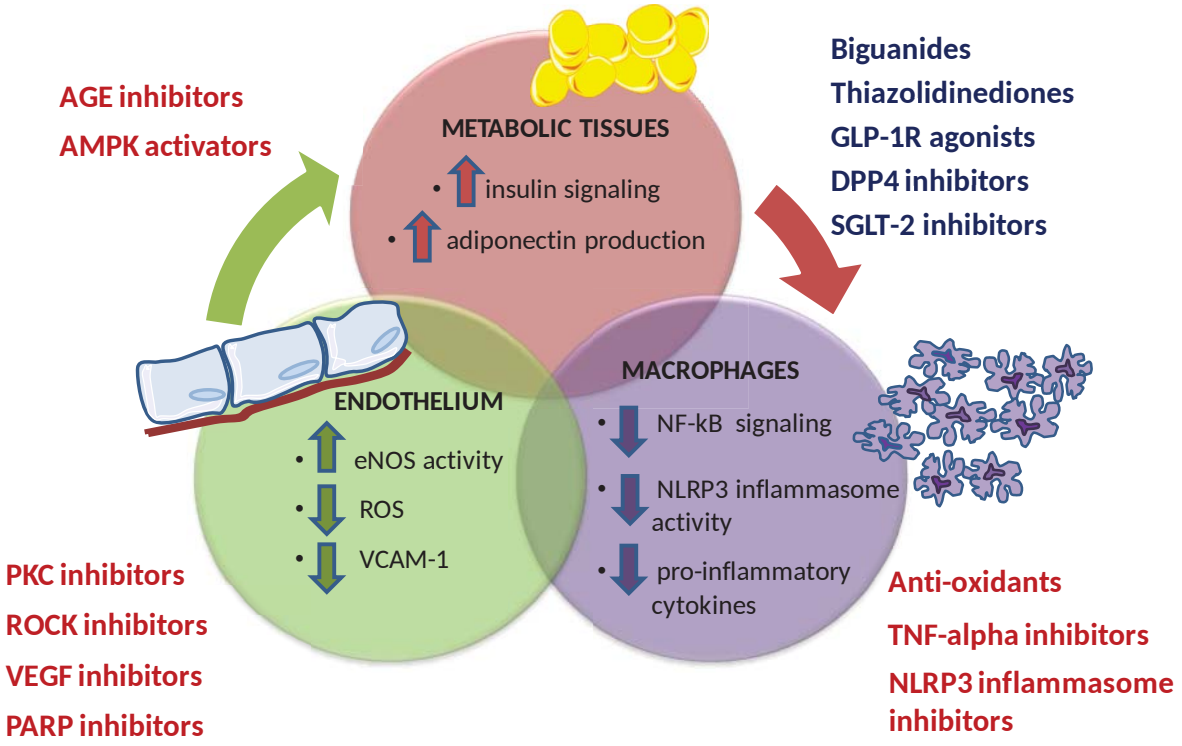

Figure 2. Current (BLUE) and perspective (RED) antidiabetic drugs may exert beneficial vascular effects by targeting several cross-talk mechanisms linking metabolic abnormalities, inflammatory response and endothelial dysfunction.

NEW STRATEGIES FOR TREATMENT OF DIABETES AND THEIR IMPACT ON ENDOTHELIAL DYSFUNCTION

The mounting understanding of pathophysiological mechanisms concurring to endothelial dysfunction in diabetes has undoubtedly multiplied the number of potential therapeutic targets for preventing or delaying its vascular complications. Among emerging strategies, molecules targeting inflammatory conditions, AGEs formation, oxidative stress or disrupted intracellular metabolic signalings may independently improve various aspects of diabetic endothelial dysfunction and are foreseen as add-on treatments. For the majority of these new molecules their possible use in clinical practice is still far from being achieved; nevertheless, they may represent the rationale alternative/additional approaches to treat or prevent vascular complications of diabetes (Figure 2).

Anti-inflammatory drugs - Increasing evidence suggests that metaflammation plays an important role in the pathogenesis of diabetes-related vascular complications, therefore suggesting that targeting inflammation may ameliorate diabetes, preventing its progression and delaying vascular complications. This concept is supported by the notion that current drugs including insulins, statins and metformin may improve diabetic symptoms by alleviating systemic and tissue-specific inflammation [178]. Since the effects of immunomodulatory treatments are not limited to tissues involved in disease pathophysiology and might have unwarranted side effects, the added value of using specific immunomodulatory treatments needs to be confirmed. Nevertheless, for some of these drugs including anti-TNFα antibody infliximab, or anti-IL1β receptor antagonist anakinra, a number of experimental and observational findings suggest their possible role on endothelial protection [92,179,180]. At present, well-designed clinical studies are still missing.

NLRP3 inflammasome inhibitors - Dietary phytochemicals, mainly flavanols, have been demonstrated to exert their vascular beneficial effects by multiple mechanisms (see below paragraph

on ***Polyphenols***). However, most recent evidence from the literature demonstrates that inhibition of the NLRP3 inflammasome may significantly contribute to the beneficial effects exerted by few of them [181]. For instance, the B-type procyanidins (PCB) have been found to suppress the activity of NLRP3 inflammasome on endothelium via the inhibition of AP-1 pathway, a transcriptional machinery involved in endothelial production of pro-inflammatory adhesion molecules and chemokines [182]. Similarly, corosolic acid, a natural triterpenoid with antioxidative activity, is supposed to protect endothelial homeostasis by suppressing NLRP3 inflammasome activation and preventing mitochondrial damage [183]. The epigallocatechin-3-gallate (EGCG), the most abundant active polyphenolic component of green tea, is capable of inhibiting NLRP3 inflammasome via enhancing the Nrf2 antioxidant pathway [184]. Moreover, the protective effects of astragaloside IV and cycloastragenol – both contained in Astragalus membranaceus Moench (Fabaceae) - against endoplasmic reticulum stress-induced apoptosis are thought to be mediated via NLRP3 inflammasome inhibition in endothelial cells [176,185]. NLRP3 inflammasome inhibition has also been proposed as new pivotal mechanism contributing to endothelial protective function of rutin, a flavonoid that can be obtained from different dietary sources [186]. Another group of natural anthocyanin derived from purple sweet potato, named purple sweet potato color (PSPC), seems to attenuate atherosclerotic progress in an insulin-resistant mice model by suppressing premature senescence of endothelium throughout inhibition of NLRP3 inflammasome [187].

Despite these interesting experimental findings, so far, the selective mechanism(s) underlying their inflammasome-suppressing effects remain largely unclear. Thus, the described activities could be due to interferences up- or downstream of inflammasome activation, and the availability of selective NLRP3 inflammasome inhibitors is an essential prerequisite to include the NLRP3 inflammasome in the list of potential pharmacological target for endothelial protection. At present, efficacious NLRP3 inflammasome inhibitors are still under development. We recently contributed to characterize the cardiovascular effect of the small molecule INF4E, one of the few compounds that has been demonstrated to directly target the NLRP3 inflammasome and inhibit the ATPase activity of NLRP3 required for its activation [97]. Similar cardiovascular protective effects have been recently documented by using another small molecule which prevents the formation of the NLRP3 inflammasome complex in cardiomiocytes, thus ameliorating cardiac function after ischemia/reperfusion injury [188]. Although there are clear indications that these compounds exert their cardioprotective effects against myocardial ischemia/reperfusion injury via a specific effect on NLRP3 inflammasome, the exact mechanism of action has still to be clarified and further insights are needed to demonstrate potential direct endothelial effects. However, the ability of members of the NLRP3 inflammasome protein complex to target molecular and cellular pathways involved in both metabolic and cardiovascular diseases suggest that selective pharmacological modulation of NLRP3 inflammasome has the potential to exert synergistic effects in the control of metabolic disorders and related cardiovascular complications. The prospective clinical relevance of this strategy is also supported by recent investigations on the influence of genetic variability in inflammasome on long-term cardiovascular complications in diabetic patients. A few studies have reported a relationship between NLRP3 genetic polymorphisms and development of type 2 diabetes [189,190] and, most notably, a specific polymorphic NLRP3 allele has been reported to be associated with increased risk for development of macrovascular complications in subjects with long-term diabetes [191].

AGE inhibitors – The renowned deleterious effects of AGEs on endothelial function and vessel structure has prompted numerous studies on molecules with promising blocking effects on AGE formation and AGE-receptor (RAGE)-mediated activity, or acting to prevent or disrupt AGE–protein cross-links (reviewed in [192-194]). Aminoguanidine is able to inhibit the formation of AGEs by interaction with and quenching of dicarbonyl compounds. Despite the reported *in vivo* ability to attenuate the formation of diabetes-induced AGEs and consequently reduce the extent of cross-linking of connective tissue proteins in the arterial wall [195], the unfavorable risk/benefit ratio discourages the use of aminoguanidine in the clinical setting [196, 197]. Another compound, alagebrium chloride (ALT-711), which cleaves AGEs and protein cross-links thereby facilitating AGEs

clearance, appears to have a more encouraging safety profile; however, protective effects on endothelial function have been not consistent in patients with isolated systolic hypertension [198], atherosclerosis [199], or chronic heart failure [200]. Although B vitamins (pyridoxamine, thiamine and its derivative benfotiamine) and pyridoxamine analogue ALT-946 may improve endothelial dysfunction [201] and prevent AGE-related complications through inhibition of AGE-dependent oxidative damage [202], some side effects on kidney function and creatinine levels [203,204] together with uncertainty on overall beneficial effects makes the clinical utility of these drugs still controversial [205].

PKC inhibitors – Impaired endothelium-dependent vasodilation secondary to hyperglycemia may be significantly improved by administration of PKC-β inhibitors [206]. Based on this observation ruboxistaurin, a selective PKC-β inhibitor, has been proposed to improve retinal blood flow distribution and decrease macular edema in diabetic patients [207,208]. In addition, treatment with this molecule has been show to ameliorate diabetic peripheral neuropathy without significant adverse effects [209]. Promising results were obtained in two combined phase III clinical trials assessing the ability of ruboxistaurin to reduce visual loss of 50% above standard care [210]. More recently, ruboxistaurin has been proposed as a potential treatment for reducing atherosclerotic plaques in diabetic patients. Since PKC activation promotes endothelial dysfunction by de-regulating IL-18/IL-18BP pathway, leading to increased VCAM-1 expression, monocyte/macrophage adhesion, and accelerated atherosclerotic plaque formation, inhibition of PKC by ruboxistaurin may represent a potential new mechanism to ameliorate endothelial dysfunction in diabetic patients [211].

VEGF inhibitors – Activation of tyrosine kinase receptors VEGFR-1 and VEGFR-2 [212] by increased levels of VEGF is associated with neovascularization in proliferative diabetic retinopathy as well as diabetic macular edema (DME) [213]. Strategies to inhibit the action of VEGF have to consider that VEGF activity is vital in processes such as angiogenesis in the myocardium and wound healing [214]. Therefore, although systemic anti-VEGF treatments are approved and used for other clinical conditions, their serious side effects in the context of diabetes must be carefully evaluated [215]. On the other hand, intraocular administration of VEGF inhibitors such as ranibizumab, bevacizumab, pegaptanib, and aflibercept has been approved for treatment of diabetic retinopathy. Ranibizumab is a recombinant antigen-binding Fab fragment of humanized anti-VEGF monoclonal antibody with a high ability to penetrate through the retina. At present, ranibizumab is the only compound approved for the treatment of visual loss due to DME [216]. Extended pharmacovigilance studies, however, are required to confirm the long-term ocular and systemic safety of ranibizumab treatment in patients with DME [217]. Bevacizumab, initially developed for intravenous treatment of metastatic colorectal cancer, has been adapted for off-label use in an intraocular administration [218] and it seems effective in decreasing retinal, disc and iris neovascularization in diabetic patients with proliferative retinopathy and macular edema [219] [220,221]. Debates remained in the past years on whether bevacizumab is superior to ranibizumab in terms of potency, efficacy and safety (reviewed in [221]). Results from the Diabetic Retinopathy Clinical Research (DRCR) phase II and Bevacizumab Or Laser Therapy (BOLT) studies showed favorable effects of intravitreal bevacizumab administration for the treatment of diabetic ocular neovascularization [222,223].

PARP inhibitors - The overactivation of the nuclear enzyme poly (ADP ribose) polymerase 1 (PARP-1) is implicated in acute endothelial dysfunction of diabetic vasculature [224,225]. Therefore, pharmacological inhibition of PARP-1 appears a potential strategy to approach diabetic vascular complications [226]. Experimental studies indicate a potential beneficial role of PARP-inhibition in diabetic retinopathy [227] and in coronary arteriole dysfunction of db/db mice [228]. The therapeutic effects of PARP-1 inhibitors are currently evaluated in clinical studies as potential candidates in cancer or cardiovascular diseases including cardiovascular complications of diabetes [229].

ROCK (Rho-associated kinase) inhibitors - The RhoA/ROCK signaling pathway mediates vascular smooth muscle contraction, downregulates eNOS gene expression and reduces protein kinase B/Akt activation, therefore decreasing eNOS phosphorylation and catalytic activity [230].

Elevated levels of peroxynitrites have been recently associated to an increased RhoA activity, largely responsible for vascular dysfunction in experimental diabetes [231]. On the same line, diabetes-induced endothelial aortic dysfunction is improved in ROCK knockout mice [232]. ROCK inhibitors such as Y-27632 and fasudil have shown promising therapeutic advantages on cardiovascular diseases including atherosclerosis, pulmonary and systemic hypertension and chronic heart failure [233,234], as well as significant beneficial effects on diabetic endothelial dysfunction of retinal [235], coronary [236] and intrarenal arteries [237]. In addition, fasudil has been able to limit TNF-alpha-mediated ICAM-1 expression and eNOS dephosphorylation in diabetic microvasculature [238]. Newer ROCK inhibitors with higher specificity among ROCK isoforms might represent potential therapeutic approaches to treat vascular complications associated to diabetes [239,240].

AMPK activators – AMPK is a serine/threonine protein kinase which plays a major role in regulating cellular and metabolic homeostasis, insulin sensitivity and mitochondrial function [241]. AMPK, activated in response to a variety of metabolic mediators, is known to regulate endothelial function and eNOS activity [242] and may contribute to ameliorate vascular endothelial function by suppressing diabetes-enhanced degradation of GTP-cyclohydrolase [243]. AMPK also suppresses inflammation, and very recently it has been reported that pharmacological activation of AMPK inhibits the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway by phosphorylating two residues (Ser^{515} and Ser^{518}) within the Src homology 2 domain of JAK1 [244]. Decreased AMPK activity has been found associated to endothelial dysfunction, apoptosis and altered lipid metabolism in aortic endothelium of obese rats [245]. Various natural compounds such as resveratrol, berberine and α-lipoic acid are able to activate AMPK *in vivo* and *in vitro*, resulting in beneficial vascular effects [246]. Numerous pharmacological agents currently used for the treatment of diabetes including biguanides, thiazolidinediones and GLP-1R agonists are able to indirectly activate AMPK [247]. Other pharmacological AMPK activators such as A-769662 and 5-Aminoimidazole-4-carboxamide roboside (AICAR) already tested in animal studies, are unlikely to be used in patients with diabetes or metabolic syndrome due to poor bioavailability and short half-life [246]. AMPK-activated signaling is involved in cardiac and vascular protective effects of Ad [46,248], whose reduced levels under diabetes are improved by numerous natural products such as fish oil, linoleic acid, green tea extracts and pharmacological agents including statins, renin-angiotensin system blockers, PPAR-α agonists and PPAR-γ agonists [40]. Despite the well-known beneficial effects of Ad, no exogenous Ad-based drugs have been developed so far [249]. In part, this may be due to concerns (see paragraph on ***Adiponectin***) related, for example, to the proteic nature of Ad that renders its oral administration ineffective, or to the unclear role of several multimeric Ad isoforms. Thus, therapeutic strategies aimed to increase endogenous Ad levels or activity are currently pursued to prevent diabetes-linked cardiovascular complications. Recently, orally active AdipoR1 and AdipoR2 agonists have been tested in animal models with promising results [250].

Anti-oxidants - Anti-oxidants is a general term to indicate a heterogeneous group of synthetic or natural substances which may potentially counteract oxidative stress by direct radical scavenging or indirect upregulation of endogenous enzymes and cytoprotective proteins. Anti-oxidant molecules may protect from endothelial dysfunction by re-coupling eNOS activity, reduce superoxide production, increase the activity of superoxide scavenging enzymes, or decrease vascular NAD(P)H oxidase activity [251,252]. Novel antioxidant therapies aiming to restore production of endothelial-derived NO or to act as endogenous antioxidant enzymes may represent a more targeted strategy focusing specifically on the mechanisms implicated in diabetes-induced endothelial dysfunction (reviewed in [240].

-Vitamin C and Vitamin E - Despite high expectation, administration of traditional antioxidants such as ascorbic acid (vitamin C) or tocopherol (vitamin E) have provided disappointing outcomes in clinical studies. Oral treatments with vitamin C or vitamin E have given controversial results on postprandial endothelial dysfunction and amelioration of forearm

vasodilation in diabetic patients [253-256]. In part, these frustrating results may be explained by the non-selective scavenging properties of vitamin C and E, which probably interfere with physiological important signaling mediated by ROS. Recent experimental studies have ascribed the protective effects of vitamin E to reduced oxidative stress and apoptosis in experimental diabetic cardiomyopathy and to decreased Ox-LDL mediated oxidative stress and vascular muscle cell proliferation in aortic wall [257,258]. Clinical studies suggest that a combination of vitamin C and insulin or a simultaneous infusion of GLP-1 and vitamin C may help to normalize endothelial dysfunction and reduce both oxidative stress and inflammation in diabetic patients [259,260], but the antioxidant potential of vitamin C and E in type 2 diabetic complications is still controversial [261-263].

- ***Polyphenols*** - Polyphenols contained in fruits, vegetables, and beverages are well known for their anti-oxidant properties [264]. Scientific interest in polyphenols as therapeutic agents is constantly increasing, and results from several experimental studies suggest that these compounds may improve endothelial function by multiple mechanisms related, but not limited, to the ability to increase eNOS expression and prostacyclin production, or to inhibit ET-1 and endothelial NADPH oxidase activity, or via more complex intercellular activities that reduce matrix metalloproteinase (MMP) activation, inhibit vascular cells migration and proliferation, and modulate angiogenesis. Polyphenols contained in cocoa, purple grape juice, red wine, black and green tea, coffee and berry have also shown the ability to acutely and chronically inhibiting platelet activation and aggregation. Moreover, for flavanols and flavonols, prevention of vascular injury has been proposed to involve counterregulation of AGE-mediated toxicity, low density lipoprotein oxidation, or inhibition of inflammatory responses (see [265,266] for complete review). Recent studies indicate that resveratrol is able to reverse the effects of hyperglycemia on mitochondrial function in endothelial cells, exerting protective actions in the early diabetes-associated endothelial dysfunction [267]. However, many studies reporting biological effects of food polyphenols are limited by the insufficient elucidation of the molecular, cellular, and physiological mechanisms underlying their effects. Obstacles in this field may include non-specific effects of polyphenols with pleiotropic activities, and complex interference among distinct active principles from the same food or beverage. In addition, based upon the structure of the particular polyphenol and the cellular redox context, most of these molecules may disclose both anti-oxidant and/or pro-oxidant properties. Finally, effects of polyphenols are highly dependent on cell type, stress conditions, and concentrations reached at the site of action. Thus, the clinical applicability of effects observed *in vitro* must be proven, since absorption through the gut may significantly reduce bioavailability and tissue concentration *in vivo*. Clinical studies addressing these issues and demonstrating the potential benefits of polyphenols are still awaited.

CONCLUSIONS

In the clinical practice, management of diabetes, obesity and cardiovascular risk factors is not always integrated. Nevertheless, the close association between metabolic disorders and a cluster of cardiovascular disturbances underscores the importance of a jointed approach to prevention and treatment. Experimental and clinical studies have progressively increased our understanding on molecular mechanisms underlying the tight and reciprocal cross-talk between hemodynamic and metabolic regulation. The role played by the endothelium on cardiovascular risks associated to diabetes and obesity may offer an integrative point of view: not only endothelial dysfunction might represent a potential unifying target of current and perspective treatments, but also provide a potential tool to assess the effectiveness of therapy. In this review, we have described the large body of data associating endothelial dysfunction to the pathogenesis of vascular complications in diabesity, focusing on inflammatory signaling as a common pathogenetic mechanism. Based on current evidence, treatments aiming at reducing glucotoxicity and lipotoxicity, simultaneously

improving metabolic homeostasis and inflammatory reaction, may effectively delay the progression of endothelial dysfunction and reduce the risk of cardiovascular events in the majority of diabetic patients. Concomitantly, the inter-individual susceptibility to diabetes and obesity, as well as the recognition of individual response to pharmacotherapy due to polymorphisms in genes encoding drug-metabolizing enzymes, transporters, receptors and signal transduction molecules is opening new roads to comprehend the variability existing in clinical outcomes of drugs used in diabesity. Overall, the choice of treatment for patients with diabesity should take into account the specific characteristics of the patient, the disease and the medication, aiming to a true personalized medicine. In this respect, the identification of intracellular signaling pathways and soluble mediators acting on metabolic, inflammatory and vascular cells has significantly broaden the spectrum of therapeutic opportunities to treat micro- and macrovascular complications of diabetes. Despite clinical substantiation is still far away, the current awareness of the key role played by the endothelium and its influence on vascular inflammation may represent an important step to develop promising new strategies to improve vascular dysfunction in diabesity.

FIGURE LEGENDS

Figure 1. The vicious circle linking metabolic abnormalities and inflammatory signaling to endothelial dysfunction in diabetes.

Figure 2. Current (BLUE) and perspective (RED) antidiabetic drugs may exert beneficial vascular effects by targeting several cross-talk mechanisms linking metabolic abnormalities, inflammatory response and endothelial dysfunction.

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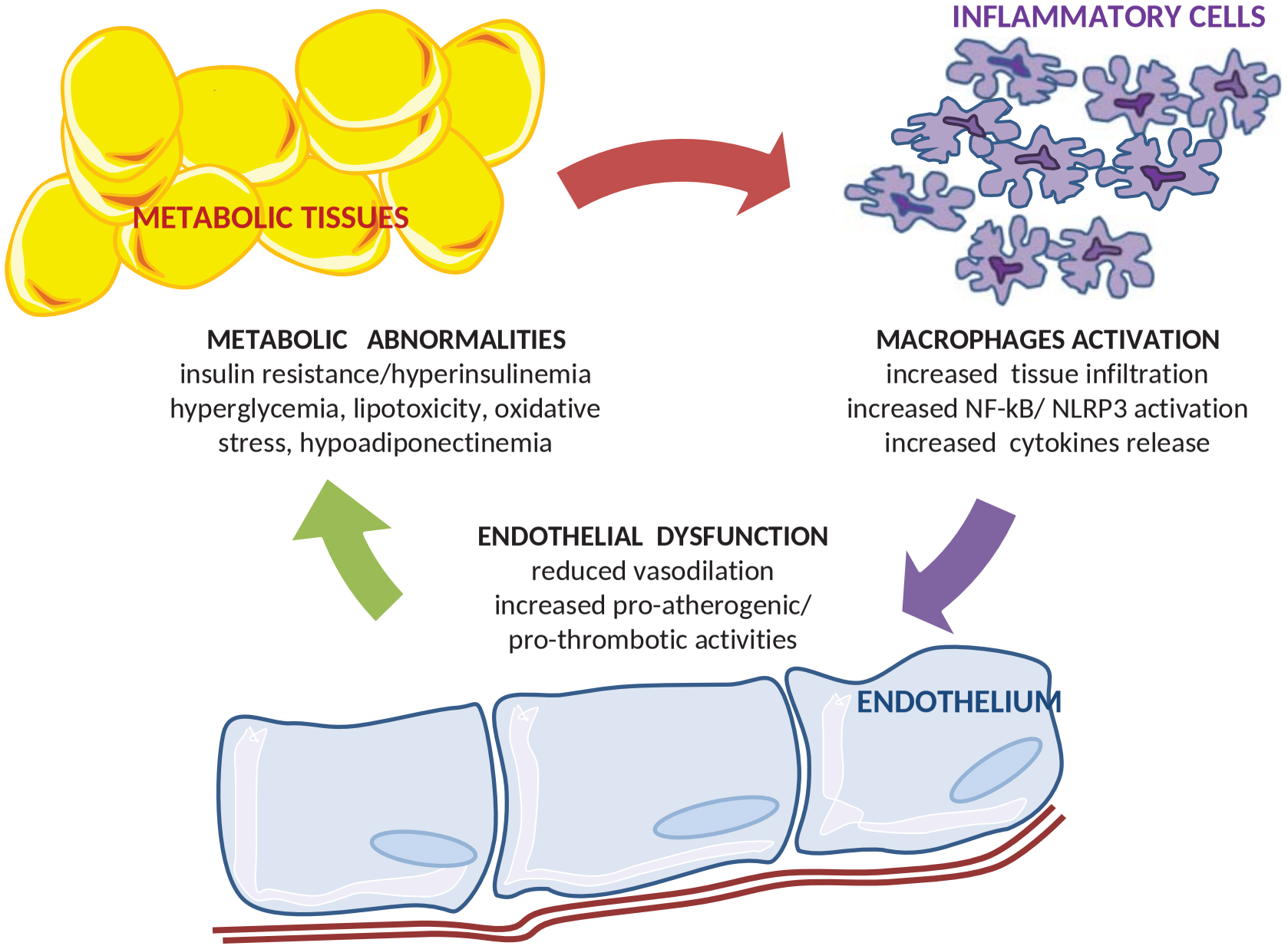

FIGURE 1. The vicious circle linking metabolic abnormalities and inflammatory signaling to endothelial dysfunction in diabesity

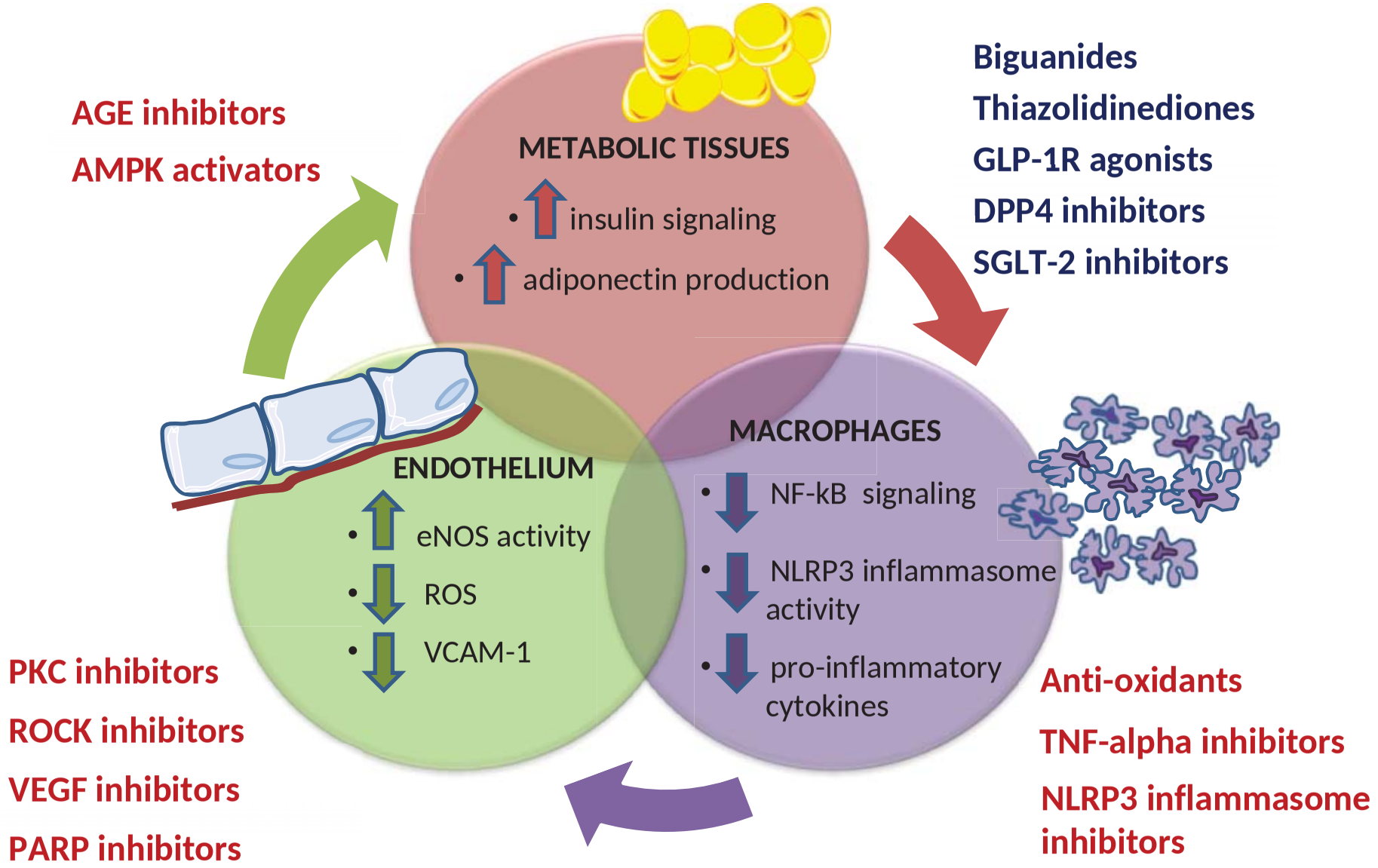

FIGURE 2. Current (BLUE) and perspective (RED) antidiabetic drugs may exert beneficial vascular effects by targeting several cross-talk mechanisms linking metabolic abnormalities, inflammatory response and endothelial dysfunction